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The goal of this project is to determine whether bone mineral density (assumed to be an integrated marker of sex steroid hormone exposure) is a risk factor for prostate cancer; and (2) to identify prostate cancer susceptibility alleles among genes in the sex steroid pathway. To address these aims, we are undertaking a case-control study of African American and Caucasian men in Pittsburgh, PA and Baltimore, MD. Cases are 100-150 African American and 150 Caucasian men with histologically-confirmed prostate cancer. Controls are age and race frequency-matched men who have a PSA <3.0 ng/mL. Hip, spine, and total body BMD is measured by Dual energy X-ray Absorptionmetry (DXA). Blood is used to obtain DNA. Polymerase Chain Reaction (PCR) techniques will be used to determine allelic distributions of genotypes for sex steroid metabolism, biosynthesis and action genes. Risk factor data are obtained by an in-person interview. Pathology information will be collected using standardized medical abstraction and all pathology will be confirmed by a central pathologist. Upon completion recruitment and data collection, we will evaluate the role of BMD and candidate genotypes in prostate cancer risk by race. We will further examine the interaction between BMD and genotypes to evaluate the hormonal environment - gene interaction and its effect on prostate cancer risk.

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INTRODUCTION:

The goal of this project is to determine whether bone mineral density (assumed to be an integrated marker of sex steroid hormone exposure) is a risk factor for prostate cancer; and (2) to identify prostate cancer susceptibility alleles among genes in the sex steroid pathway. To address these aims, we are undertaking a case-control study of African American and Caucasian men in Pittsburgh, PA and Baltimore, MD. Cases are 100-150 African American and 150 Caucasian men with histologically-confirmed prostate cancer. Controls are age and race frequency-matched men who have a PSA < 3.0 ng/mL. Hip, spine and total body BMD is measured by Dual-energy X-ray Absorptiometry (DXA). Blood is used to obtain DNA. Polymerase Chain Reaction (PCR) techniques will be used to determine allelic distributions of genotypes for sex steroid metabolism, biosynthesis and action genes. Risk factor data are obtained by an in-person interview. Pathology information will be collected using standardized medical abstraction and all pathology will be confirmed by a central pathologist. Upon completion recruitment and data collection, we will evaluate the role of BMD and candidate genotypes in prostate cancer risk by race. We will further examine the interaction between BMD and genotypes to evaluate the hormonal environment – gene interaction and its effect on prostate cancer risk.

BODY:

In this section, we describe our accomplishments according to the Work Plan originally approved:

Task 3 Recruiting of Subjects and Obtaining of Data, Months 6-30

- **see tables 1-3 for summaries to date. In short, we have recruited 116 (77%) Caucasian cases, 69 (46%) Caucasian controls, 17 (11%) AA cases and 7 (4.7%) AA controls.**

Task 4 Performance of Laboratory Assays, Months 12-31:

a. Isolate DNA from blood samples
   - **DNA was isolated on 88 case subjects thus far**

b. Assay samples (600) to detect sex steroid related genetic polymorphisms and record results on study forms
   - **The following genotyping assays were performed:**
     a. AIB1/SRC3 - steroid receptor coactivator 3; CAG (glutamine) repeat polymorphism
     b. CYP11A - cholesterol side chain cleavage enzyme; pentanucleotide repeat [(TTTTA)n] in the promoter
     c. SHBG - pentanucleotide repeat [(TAAAA)n] located in an Alu sequence at the 5' boundary of the promoter
     d. CYP19 - aromatase; intronic tetranucleotide repeat [(TTTA)n]
     e. HSD11B1 - 11-beta hydroxsteroid dehydrogenase; a CA repeat

c. Retest a subset of specimens (60) to validate the laboratory results

Task 5 Data Entry, Months 12-32:

a. Enter, verify and clean interview, anthropometric, physical activity, pathology, DXA, and laboratory assay data via the PoP computerized data entry system
   - As reported last year, we implemented the questionnaire in TeleForm, so that data entry is ongoing. All data is entered when a subject is interviewed.

Task 6 Interim Analyses of Data, Months 18-30 :

a. Perform interim statistical analyses of data periodically

- See tables 1-3 for data

Overall Study Progress:

To date, we are making excellent progress in recruiting Caucasians in Pittsburgh. We are ahead of recruitment schedule. Ms. Overberger, the study coordinator, resigned in July. We have hired a new coordinator, Ms. Gail Engleka. She will begin on October 1, 2003. Despite this delay, we are almost completed with case recruitment in Pittsburgh and will finish with control recruitment by the end of the study period.

Pittsburgh Progress:

We obtained IRB approval from the University of Pittsburgh to commence recruitment of subjects in Pittsburgh. Recruitment began in February 2002. Cases are recruited from all newly diagnosed cases of prostate cancer seen in the practice of Dr. Joel Nelson. Controls are men who have participated in a population-based prostate cancer screening trial and are frequency matched to cases by age and race. The summary of recruitment From January 2003 through August 2003 is in Table 1. We recruit approximately 4 men per week in Pittsburgh (2-3 cases, 1-2 controls), which is ahead of the anticipated recruitment schedule. Because interview data is scanned in weekly, we are able to provide interim data analyses. Table 2-3 summarizes the baseline data on all recruited subjects through August 2003.

Baltimore Progress:

We obtained IRB approval from the University of Maryland in August, 2002. This was later than anticipated and has put our recruitment behind. Moreover, the DOD Human Subjects Committee has not yet approved adding Baltimore as a site for this study and we are therefore unable to commence recruitment of African Americans in Baltimore. Our last application for approval from the DOD Human Subjects Committee was August 7, 2003.

Nonetheless, in September 2002, Drs. Modugno and Weissfeld visited the Baltimore site to meet with the Baltimore PIs (Marc Hochberg, MD and Richard Alexander, MD) and study team. A part-time Baltimore study coordinator was hired (Ms. Jennifer DeSanto) through the University of Maryland Center for Clinical Trials. Ms. DeSanto will work with Dr. Alexander's staff in the urology clinic to recruit eligible African American cases. Ms. DeSanto will work two days per week (Thursday and Friday) when the urology clinic at the Baltimore VA takes place. Ms. DeSanto is paid only for the days she works and since recruitment has not begun, we have no costs associated with Ms. DeSanto to date. Ms. DeSanto received all the study instruments and instructions. During the first 2-3 weeks of recruitment, Dr. Modugno and Ms. Engleka will go to Baltimore to provide on-site training of Ms. DeSanto (consenting and interviewing subjects).

Because of the late start of recruitment in Baltimore, Dr. Modugno has authorized that funds from Y1 and Y2 of this grant that were to be used to support recruitment in Baltimore be set aside. This will ensure the availability of funds to continue recruitment in Baltimore after the 3 year study period ends (if need be). These funds will cover the cost of the study coordinator in Baltimore and all the associated study costs (DXAs, etc).

Once we begin recruitment in Baltimore, cases will be recruited from the VA urology clinic during their weekly clinic schedule (Thursdays and Friday mornings). Controls will be recruited from Dr. Hochberg's ongoing study of BMD in African American men. Controls will be frequency matched by age to cases.

Addition of Alabama

To increase minority recruitment in a timely fashion, we have initiated a subcontract with the University of Alabama, Charlotte Mayo, PI. (Attached). We are awaiting IRB approval for the Alabama site. Once approval is obtained, we will begin recruitment at that site. We will not make any site visits until IRB approval is obtained in order to conserve funds.

Exclusion Criteria

The following are the criteria used to exclude men from participation in this study.

- <40 or >80 years of age
- Inability to consent to medical procedures.
- History of hyper or hypothyroidism, hyperparathyroidism, renal disease, or bone disorders
- History of hypogonadism
- History of Bone Disease/problems – osteoporosis, Paget's disease, osteomalacia, osteogenesis imperfecta,
- Chronic (>3 months) glucocorticoid therapy
- Use of testosterone supplementation (>3 months)
- Use of bisphosphonate supplementation (>3 months)
- Bilateral hip replacement
- Kidney or liver transplant recipient
- Previous diagnosis of cancer, except basal/squamous cell skin cancer
- trouble absorbing vit D., vit D deficiency, calcium abnormality, brittle bones
- 2 or more non-traumatic fractures over a lifetime or 1 or more non-traumatic fracture in the last year.
- For prostate cancer cases, evidence of bone metastases
- For controls, PSA levels above 3 ng/mL within the last 3 months

Data Collection

The following data are collected on all participants:

- demographics, lifestyle factors and medical history via a ½ hours in-person interview
- hip, spine and total BMD via a DXA scan. Results are abstracted onto a study form
-
- 35 ml of blood. This is used to isolate DNA for the current study. In addition, the following specimens are banked:
    - serum (8x1mL)
    - plasma (8x1mL)
    - buffy coat
    - clot
- height, weight and hip circumference (measured by study personnel during study visit). Results are recorded on a study form.

Laboratory Assays

All genotyping assays are done in the laboratory of Dr. Robert Ferrell. High molecular weight DNA will be extracted from peripheral blood leukocytes by the salting-out procedure. Polymerase Chain Reaction (PCR)

and Restriction Fragment Length Polymorphism(RFLP) techniques will be used to identify polymorphisms in the sex steroid metabolism pathway. Restriction fragment length polymorphisms are genotyped by amplification of the variable site using unique sequence flanking primers, digestion with an appropriate restriction endonuclease, resolution of the fragments on 2% agarose gels and visualization under UV light after ethidium bromide staining. Single nucleotide polymorphisms that do not alter a restriction site are assayed by a modified allele specific oligonucleotide ligation assay. Length polymorphisms are genotyped by amplification using unique sequence flanking primers, one of which is labeled with a fluorescent dye (FAM, HEX or TET; Research genetics, Huntsville, AL). The products are resolved on the ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) and the resulting gel images are analyzed using the GENESCAN software package. These protocols are standard in Dr. Ferrell's lab. Genotypes are assigned by two independent readers by directly comparing test samples to sequence-verified control samples run on the same gel. Conflicts are resolved by repeat genotyping.

We have tested the laboratory assays on a sample of specimens early in our recruitment. The assays appear to be working.

BMD Measurments

Hip, spine and total body BMD will be measured by Dual-energy X-ray Absorptiometry (DXA) using a Hologic QDR-4500A (Hologic, Inc.,Waltham, MA) in the Laboratory of Dr. Susan Greenspan. Quality control is assessed by daily quality control scans with the phantom provided by the manufacturer. We will also have a subset of scans (10%) reanalyzed by Synarc, Inc. (Bedford, MA), which provides quality control for large scale studies, including several of Dr. Greenspan's studies. All DXA results will be recorded on a standard study form for data entry.

Problems encountered and measures taken:

Minority recruitment has been very difficult. Because of the new HIPAA regulations, we are unable to identify minority cases as we originally intended in Pittsburgh; thus, our minority recruitment is very low. We are therefore relying on the minority recruitment sites for minority recruitment (Baltimore and Alabama).

The major problem we have encountered is obtaining IRB approval for this study at Baltimore and the DOD. We have already received IRB approval in Baltimore. We are working with the DOD to receive approval to add Baltimore to the study so that we can begin recruitment at that site.

Because of the substantial delay in Baltimore, we added another minority site: University of Alabama at Birmingham, Charlotte Mayo, PI. This will enable us to recruit minorities concurrently at two sites and hopefully enable us to reach our goal within the study timeline. To further ensure this goal is reached, we have requested a 1 year no-cost extension for the project. We have further had funds for the minority portion of the project (approximately $150,000), set aside.

KEY RESEARCH ACCOMPLISHMENTS:
The study is well underway with all the components in place. We forsee successfully completing recruitment of Caucasians in Pittsburgh well within the study period. We anticipate successfully completing African American recruitment, although we anticipate that this will require additional time due to the delay in recruitment in Baltimore because of the delay in receiving DOD IRB approval to add Baltimore as a site for this study.

REPORTABLE OUTCOMES:
None to date

CONCLUSIONS:
We are pleased with our progress and forsee the successful completion of this project. We are working with the DOD to receive IRB approval to begin recruitment in Baltimore. We are working with the University of Alabama to receive IRB approval at that site and to then receive DOD approval.

REFERENCES:
None

APPENDICES:
None

Table 1 CAPS Recruitment Summary for Cases 2003:

	Jan 2003	Febr 2003	March 2003	April 2003	May 2003	June 2003	July 2003	August 2003	YTD 2003
Summary of Enrollment:									
Total Number of Eligible Men:	14	8	12	8	12	14	26	12	106
Number of Men Approached:	10	8	12	8	7	12	20	10	87
** Eligible Men Not Approached:	4				5	2	4	2	17
Physician Refused:									0
Recruiter Missed:									0
Exclusionary Medical Condition:	1				4	1	4		10
Mentally Incompetent:									0
Outside Age Range:									0
Other:	3				1	1	2	2	9
Number of Men Agreeing to be Contacted:	9	8	12	7	7	10	17	9	79
Number of Men Screened:	9	8	12	7	7	10	17	9	79
Number of Men Enrolled (consented):	5	4	7	5	2	5	10	5	43
Number of Men Scheduled for Interview:	0	0	0	1	0	1	3	4	9
Number of Men Waiting for Appt:									0
Number of Men Not Enrolled***:	4	4	5	1	5	4	4		27
Number of Men Needing to be Screened:									0
Asked to be re-contacted:									0
Not yet contacted:									0
Failed contact:									0
*** Reason Screened But Not Enrolled:									0
Declined participation:	4	3	3		2	4	4		20
History of Cancer:									0
History of Exclusionary Medical Condition:									0
Unable to Give Blood:									0
Use of Exclusionary medication:									0
Other:		1	2	1	3				7

Table 2: Age and Race Distribution of Cases and Controls Recruited in Pittsburgh from 2/1/02 to 8/31/03

	Caucasian		African-American	
Age Range	Cases	Controls	Cases	Controls
40-44	2			4
45-49	2			1
50-54	18		3	1
55-59	33	38	5	1
60-64	30	21	4	
65-69	20	8	3	
70-74	10	2	2	
75-79	1			
Total	116	69	17	7

Table 3: Summary Demographic Statistics on Cases and Controls for Pittsburgh from 2/1/02 to 8/31/03

	Cases n=133	UPMC Cases n=130	Caucasian Cases n=116	AA Cases n=17	Controls n=76	UPMC Controls n=68	Caucasian Controls n=69	AA Controls n=7
Age (mean yrs)	60.11	60.09	60.01	60.82	59.17	60.54	60.51	46
Race								
Caucasian	116	116	116		69	68	69	
African-American	17	14		17	7			7
Recruitment Site								
UPMC	130		116	14	68		68	
VA	3			3	8		1	7
Education								
< 8 yrs					1	1	1	
8 to 11 yrs	2	2	2					
12 yrs or HS	28	27	21	7	14	12	12	2
post secondary	2	2	1	1	12	10	10	2
some college	19	17	12	7	14	11	12	2
college graduate	39	39	37	2	16	15	15	1
postgraduate	43	43	43		19	19	19	
Marital Status								
never married	4	4	3	1	8	6	6	2
married	115	115	103	12	59	54	55	4
widowed	3	3	3	3	3	3	3	
divorced	10	10	7	1	6	5	5	1
separated	1	1						
BMD (g/cm2) (mean)								
Hip	1.021	1.023	1.02	1.032	1.031	1.021	1.023	1.113
n	131	128	114	17	76	68	69	7
Spine Lateral	0.789	0.791	0.784	0.825	0.806	0.798	0.798	0.887
n	125	122	109	16	74	66	67	7
Spine PA	1.081	1.081	1.081	1.077	1.076	1.075	1.075	1.08
n	129	126	112	17	76	68	69	7
Total Body	1.17	1.172	1.17	1.17	1.17	1.168	1.168	1.183
n	127	124	110	17	76	68	69	7
Lean Body Mass	61.83	61.99	61.95	61.04	63.51	63.04	63.13	67.33
n	130	127	113	17	76	68	69	7
% Body Fat	25.62	25.56	25.7	25.08	26.97	26.71	26.86	28.06
n	130	127	113	17	76	68	69	7